Enterotoxins

BY

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ENTEROTOXIN

- A number of bacteria produce exotoxins that bind to the cells of the small intestines. Most of these toxins catalyze the ADP-ribosylation of host cell proteins that turn the synthesis of the metabolic regulator molecules cyclic AMP (cAMP) or cyclic GMP on and off in intestinal mucosal cells. High levels of cAMP and cGMP cause loss of electrolytes and water that results in diarrhea. Organisms producing enterotoxins include *Clostridium perfringens,and , Bacillus cereus.*

- One aspect the classification and nomenclature of these toxins must reflect is the type of cell affected :

1. Cytotoxins; produce toxic effects in many different host cells
2. Neurotoxins; affect the neurons
3. Enterotoxins affect enterocytes .

The structures and mechanisms of action of the toxins are also considered in their classification.

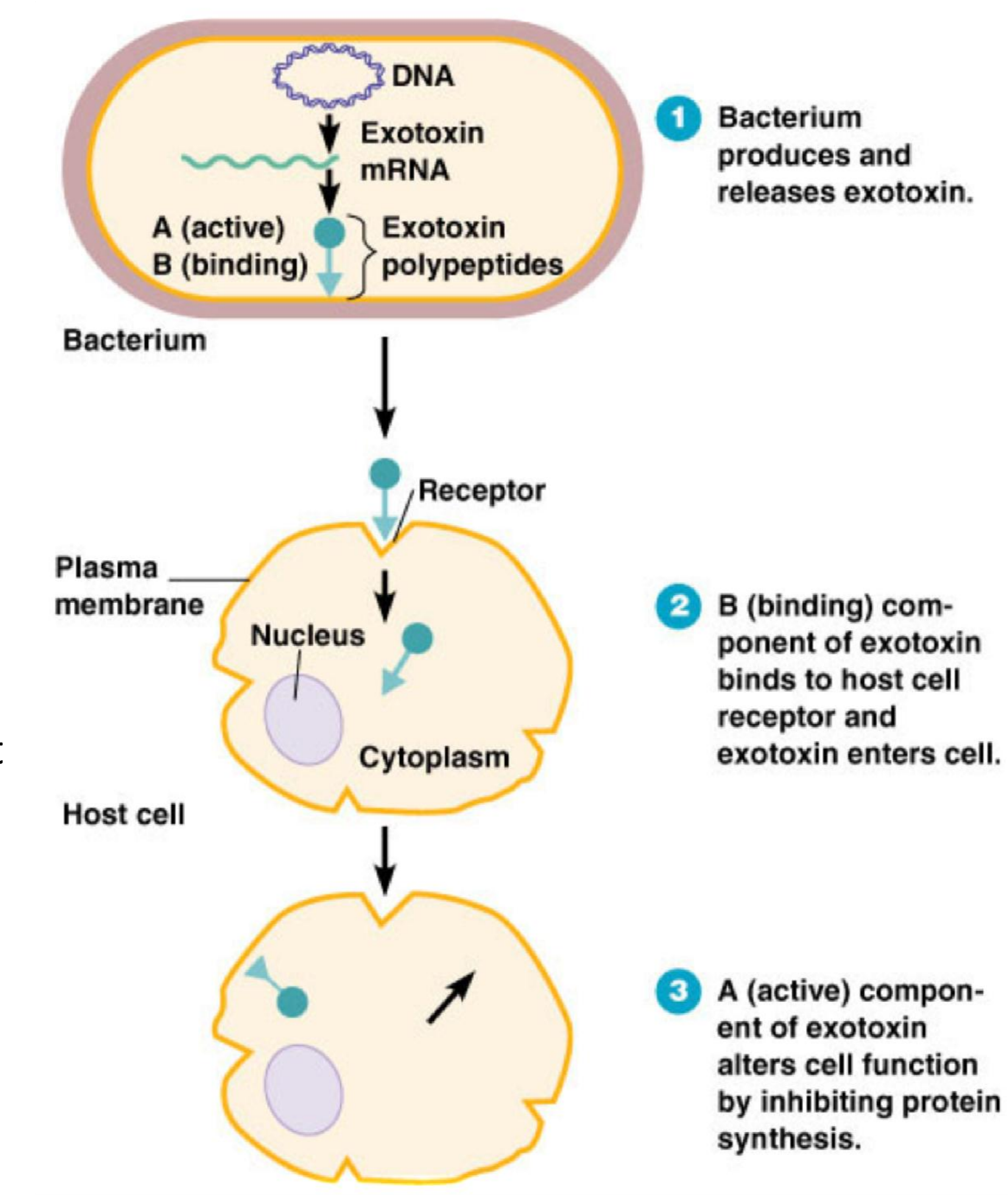
Exotoxins

1. **Cytotoxins**
   kill cells

2. **Neurotoxins**
   interfere with normal nerve impulses

3. **Enterotoxins**
   effect cells lining the G.I. Tract
   Many toxins have A-B subunit toxins or type III toxins

- **A**:
  Active Causes change in host
- **B**:
  Binding



ENTEROTOXIN

1. (not to be confused with endotoxin) is a protein toxin released by a microorganism in the intestine.

2. Enterotoxins are chromosomally encoded exotoxins that are produced and secreted from several bacterial organisms. They are often heat stable, of low molecular weight and are water-soluble.

3. Enterotoxins are frequently cytotoxic and kill cells by altering the membrane permeability of the mucosal (epithelial) cells of the intestinal wall .

4. They are mostly pore-forming toxins (mostly chloride pores), secreted by bacteria, that assemble to form pores in cell membranes. This causes the cells to die.

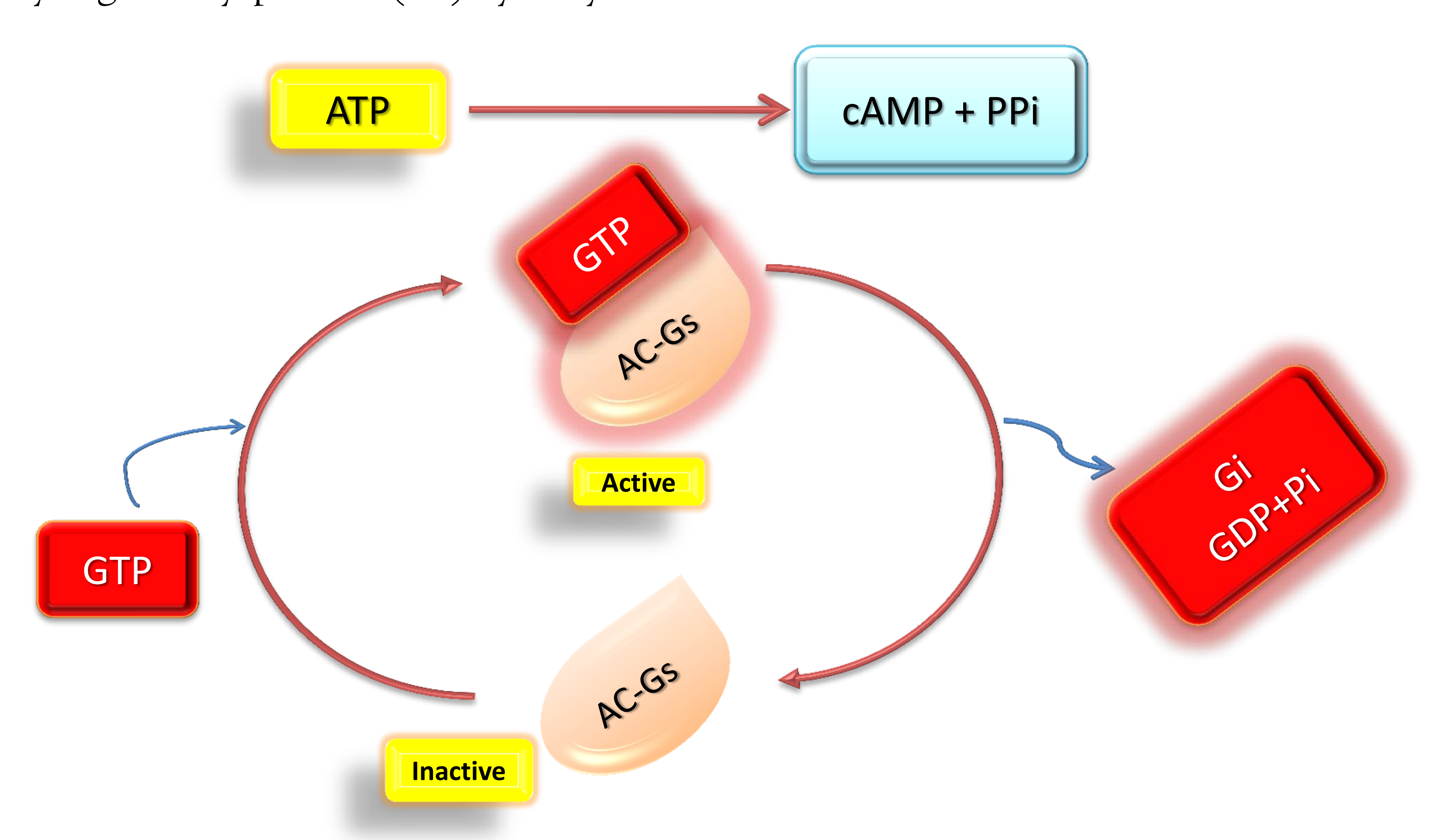
CLINICAL SIGNIFICANCE

The action of enterotoxins leads to:

1. **Increased chloride ion permeability** of the apical membrane of intestinal mucosal cells.
2. These membrane pores are activated by either **increased cAMP** or by increased calcium ion concentration intracellularly.
3. The **pore formation** has a direct effect on the osmolarity of the luminal contents of the intestines.
4. Increased chloride permeability leads to **leakage** into the lumen followed by sodium and water movement.
5. This leads to a **secretory diarrhea** within a few hours of ingesting enterotoxin. Several microbial organisms contain the necessary enterotoxin to create such an effect, such as *Staphylococcus aureus* or *E. coli.*

Normal regulation of adenylate cyclase activity in mammalian cells

Adenylate cyclase (**AC**) is activated normally by a stimulation regulatory protein (**Gs**) and Guanosine triphosphate (**GTP**); however the activation is normally brief because an inhibitory regulatory protein (**Gi**) hydrolyzes the GTP.



ORGANISMS SECRETING ENTEROTOXINS

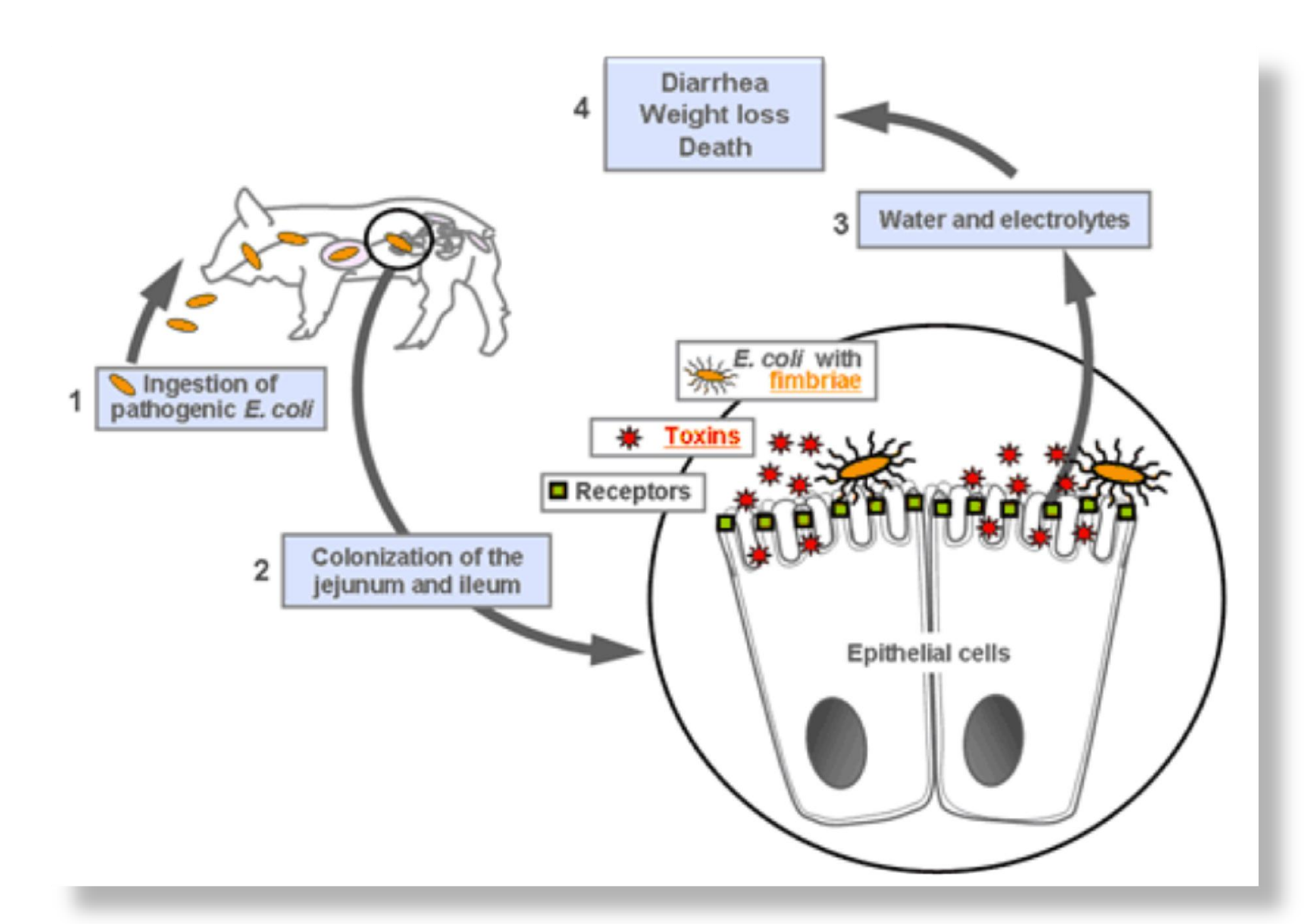
Examples of organisms secreting enterotoxins are:

Bacterial

- *Escherichia coli*
- *Clostridium perfringens Clostridium perfringens enterotoxin*
- *Clostridium difficile*
- *Vibrio cholerae Cholera toxin*
- *Staphylococcus aureus Staphylococcal Enterotoxin B*
- *Yersinia enterocolitica.*
- *Shigella dysenteriae Shiga toxin*
- *Enteric Salmonelloses*
- *Campylobacter jejuni*

Escherichia coli

- Intestinal infections are caused by the pathovars EPEC, ETEC, EIEC, EHEC, and EAggEC. EPEC and EAggEC frequently cause diarrhea in infants.
- ETEC produce enterotoxins that cause a choleralike clinical picture Enterotoxic *E. coli* (ETEC). The pathogenicity of these bacteria is due to the heat-labile enterotoxin LT (inactivation at 60 C for 30 minutes) and the heatstable toxins STa and STb (can tolerate temperatures up to 100 C.
- Some strains produce all of these toxins, some only one. LT is very similar to cholera toxin ctx .It stimulates the activity of adenylate cyclase. STa stimulates the activity of guanylate cyclase. (cGMPmediates the inhibition of Na^+ absorption and stimulates Cl^- secretion by enterocytes.) ETEC pathogenicity also derives from specific fimbriae, so-called colonizing factors CFA that allow these bacteria to attach themselves to small intestine epithelial cells, thus preventing their rapid removal by intestinal peristalsis. The enterotoxins and CFA are determined by plasmid genes. The clinical picture of an ETEC infection is characterized by massive watery diarrhea. The disease can occur at any age. Once the illness has abated, a local immunity is conferred lasting several months.
- Shiga-like toxin produced by EHEC, causing a severe form of copious, bloody diarrhea (hemorrhagic colitis) in the absence of mucosal invasion or inflammation. Serotype O157:H7 is the most common strain of E. coli that produces verotoxin
- The DNA that encodes the LT toxin is on a plasmid that can be transferred to other E. coli strains and probably to other enteric bacteria as well.


4
Diarrhea
Weight loss
Death
3
Water and electrolytes
1
Ingestion of
pathogenic E. coli
E. coli with
fimbriae
Toxins
Receptors
2
Colonization of the
jejunum and ileum
Epithelial cells

Clostridium difficile

Clostridium difficile **enterotoxin toxin** A, cytotoxin toxin B Pseudomembranous colitis (often antibotic associated). *Clostridium difficile* (Pseudomembranous Colitis) *C. difficile* occurs in the fecal flora of 1-4 %of healthy adults and in 30-50 %of children during the first year of life.

The factors that lead to development of the disease are not known with certainty. Cases of pseudomembranous colitis are observed frequently under treatment with clindamycin, aminopenicillins, and cephalosporins (hence the designation antibiotic-associated colitis), but also occur in persons not taking antibiotics.

Occasional outbreaks are seen in hospitals. The pathological mechanism is based on formation of two toxins. Toxin A is an enterotoxin that causes a dysfunction characterized by increased secretion of electrolytes and fluids. Toxin B is a cytotoxin that damages the mucosa of the colon.

Vibrio cholerae

- Vibrio cholerae holera toxin, The disease develops when the pathogens enter the intestinal tract with food or drinking water in large numbers $>10^8$. The vibrios multiply in the proximal small intestine and produce an enterotoxin.

- Cholera toxin is a multimeric protein composed of an A and a B subunit. The B subunit (consisting of five identical monomers) binds to the GM1 ganglioside receptor of cells lining the intestine.

- This toxin stimulates a series of reactions in enterocytes, the end result of which is increased transport of electrolytes out of the enterocytes, whereby water is also lost passively. Massivewatery diarrhea results in exsiccosis.

- The A subunit has two components: A2, which facilitates penetration of the cell membrane, and A1, an ADP-ribosyl transferase that ADP-ribosylates the membrane-bound Gs protein.1 Gs protein activates adenylate cyclase, which produces elevated levels of intracellular cAMP. This, in turn, causes an out flowing of ions and water to the lumen of the intestine.

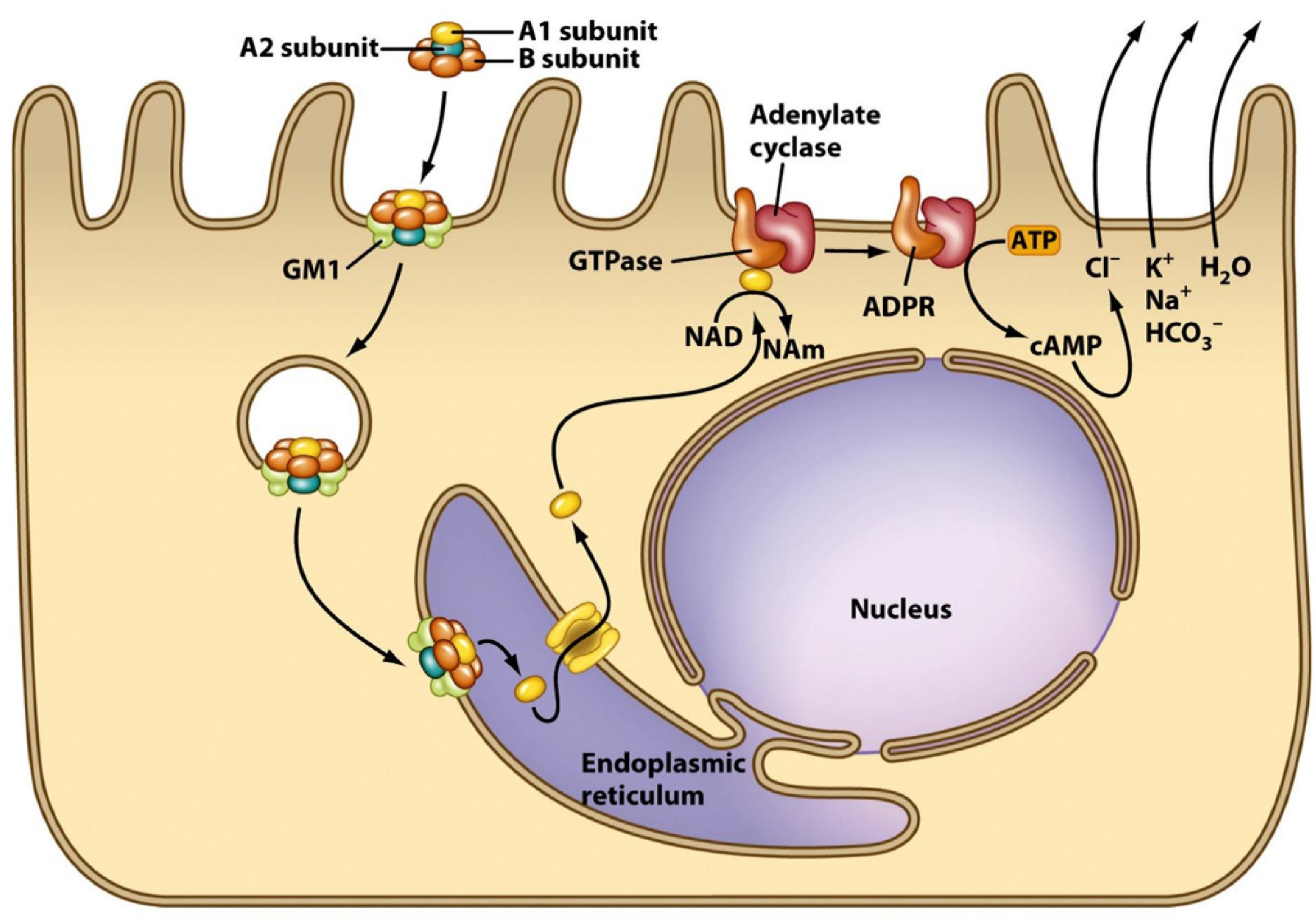
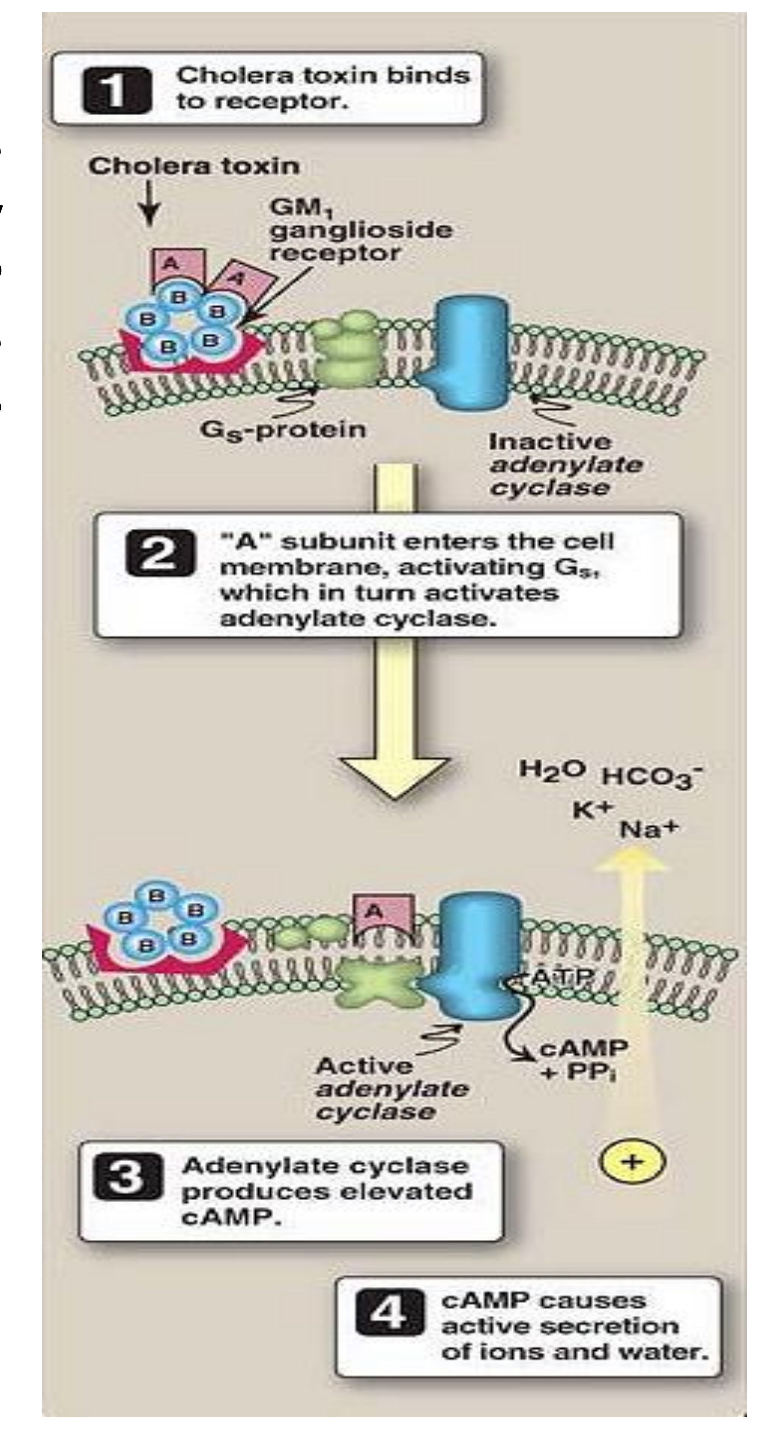
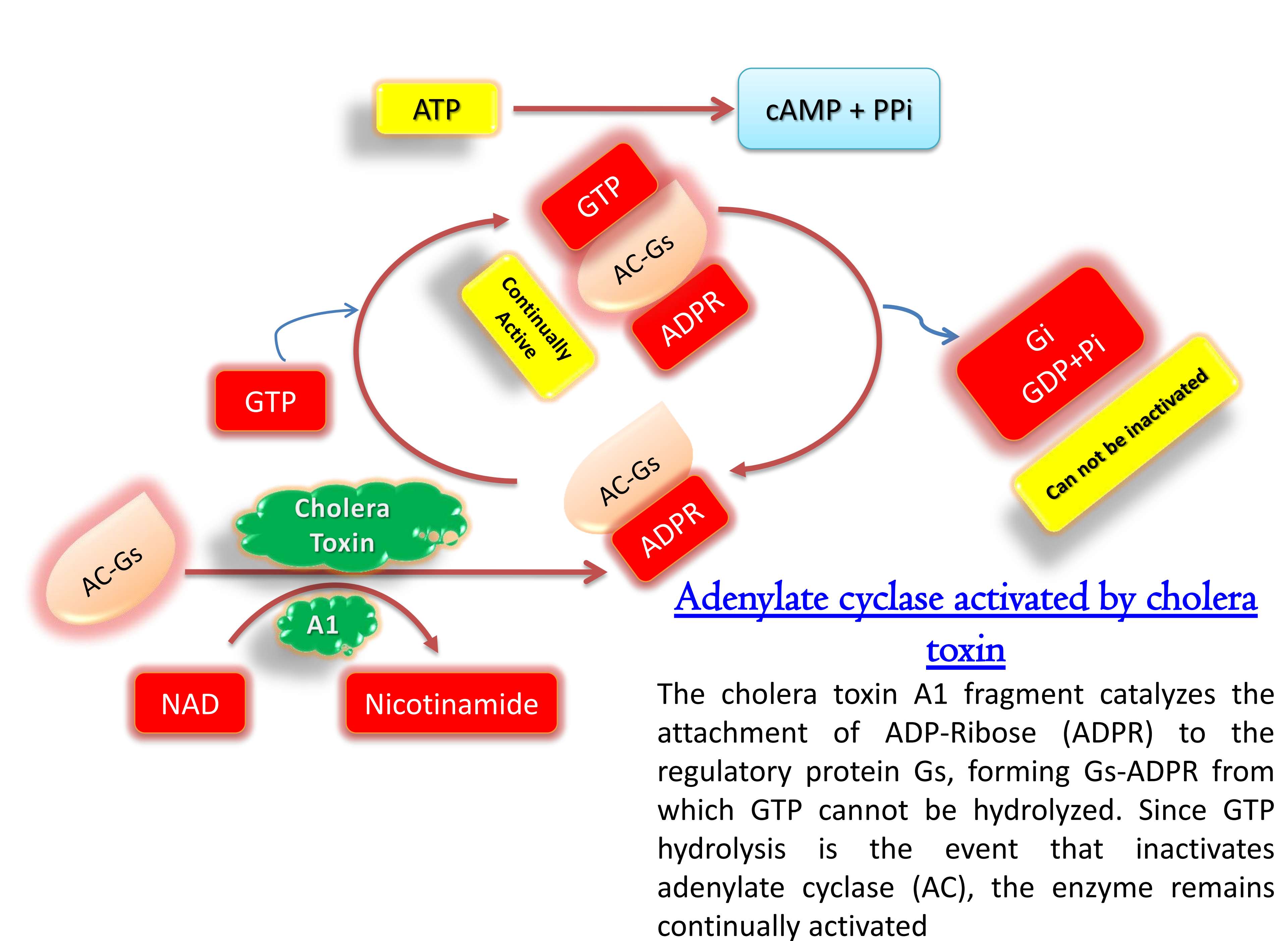


Figure 25.19a Microbiology: An Evolving Science

Adenylate cyclase activated by cholera toxin

The cholera toxin A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein Gs, forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates adenylate cyclase (AC), the enzyme remains continually activated

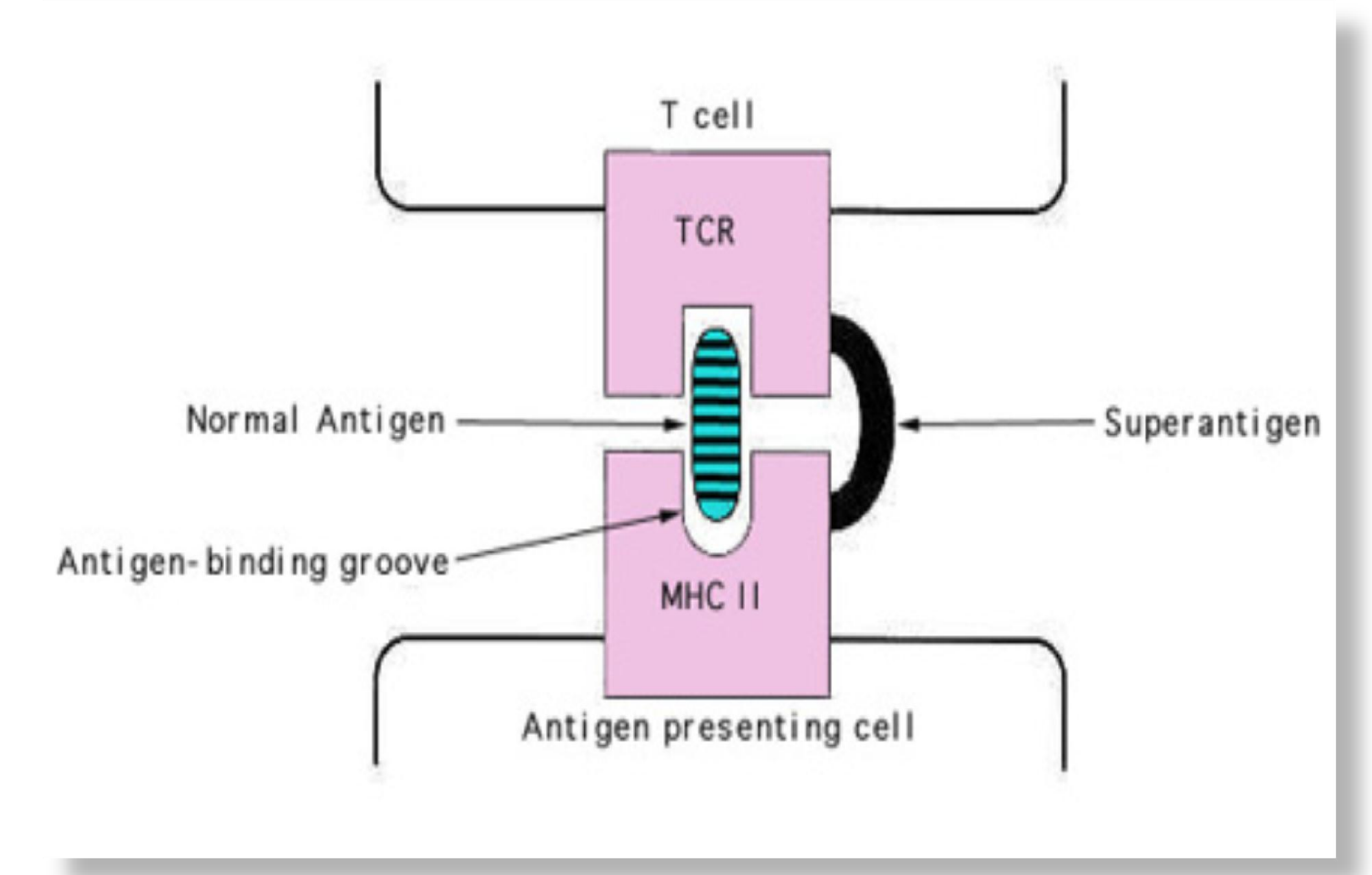


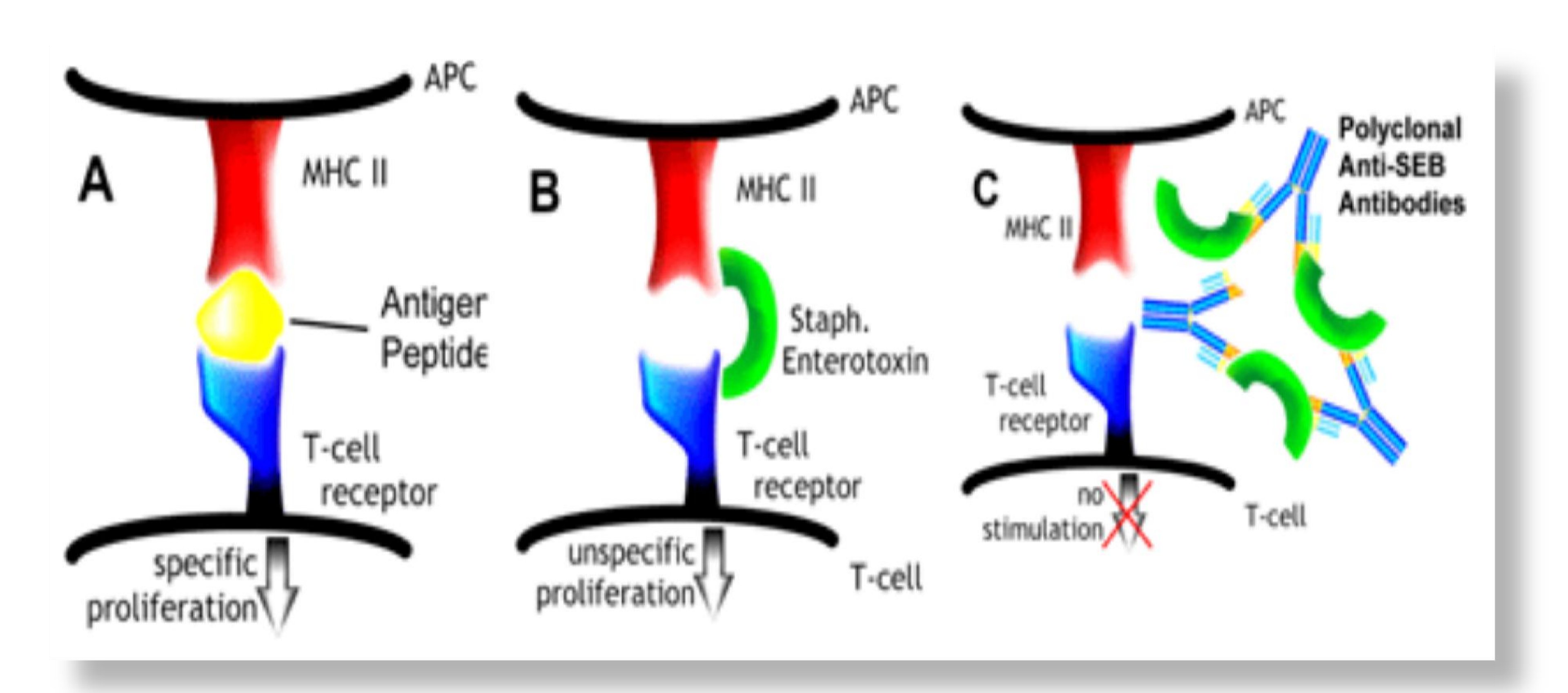

ATP
cAMP + PPi
GTP
AC-Gs
ADPR
Continually Active
GTP
Gi
GDP+Pi
Can not be inactivated
AC-Gs
ADPR
Cholera Toxin
AC-Gs
A1
NAD
Nicotinamide
Adenylate cyclase activated by cholera toxin
The cholera toxin A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein Gs, forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates adenylate cyclase (AC), the enzyme remains continually activated

Staphylococcus aureus

Staphylococcal Enterotoxin B **SEB**

1. Food poisoning symptoms can be caused by eight serologically differentiated enterotoxins (A-E, H, G, and I). These proteins are not inactivated by heating to 100 C for 15-30 minutes. *Staphylococcus* enterotoxins are superantigens .
2. Toxicoses. Food poisoning results from ingestion of food contaminated with **enterotoxins**. The onset a few hours after ingestion takes the form of nausea, vomiting, and massive diarrhea.
3. Many of the effects of SEB are mediated stimulation of T lymphocytes by the host's immune system. The toxin binds directly to the major histocompatability complex (MHC) class II proteins on target cells, subsequently stimulating the proliferation of large numbers of T lymphocytes.
4. SEB is a "bacterial superantigen" because it can form a "bridge" between the MHC II on the antigen presenting cells and the T-cell receptors on both CD4 and CD8 T cells, thereby bypassing the normal antigen processing and presenting mechanism. This bridging effect causes the release of massive amounts of cytokines, specifically interleukin 2 (IL-2), tumor necrosis factor b (TNF-b), and interferons.


T cell
TCR
Normal Antigen
Superantigen
Antigen-binding groove
MHC II
Antigen presenting cell



A
APC
MHC II
Antigen
Peptide
T-cell
receptor
specific
proliferation
B
APC
MHC II
Staph.
Enterotoxin
T-cell
receptor
unspecific
proliferation
T-cell
C
APC
MHC II
Polyclonal
Anti-SEB
Antibodies
T-cell
receptor
no
stimulation
T-cell

Yersinia enterocolitica

All of the strains isolated as human pathogens bear a 70 kb virulence plasmid with several determinants. They code for polypeptides that direct the functions cell adhesion, phagocytosis resistance, serum resistance, and cytotoxicity. Yersiniae also have chromosomal virulence genes, for example markers for invasins, **enterotoxins**, and an iron capturing system

Shigella dysenteriae *Shiga toxin*

- **Shiga toxins** are a family of related toxins with two major groups, Stx1 and Stx2, The toxins are named for Kiyoshi Shiga, who first described the bacterial origin of dysentery caused by *Shigella dysenteriae*.

- Shiga toxins act to inhibit protein synthesis within target cells . After entering a cell, the protein functions as an N-glycosidase, cleaving several nucleobase from the RNA that comprises the ribosome, thereby halting protein synthesis.

- The toxin has two subunits—designated A and B—and is one of the AB_5 toxins. The B subunit is a Pentamer that binds to specific glycolipids on the host cell.

- Following this, the A subunit is internalized and cleaved into two parts. The A1 component then binds to the ribosome, disrupting protein synthesis (A1 domain causes inactivation of **the 60S ribosomal subunit**, leading to cell death from inhibition of protein synthesis). Stx-2 400 times more toxic than Stx-1. a heat-labile exotoxin is released by *Shigella dysenteriae* that damages the mucosa and villi.

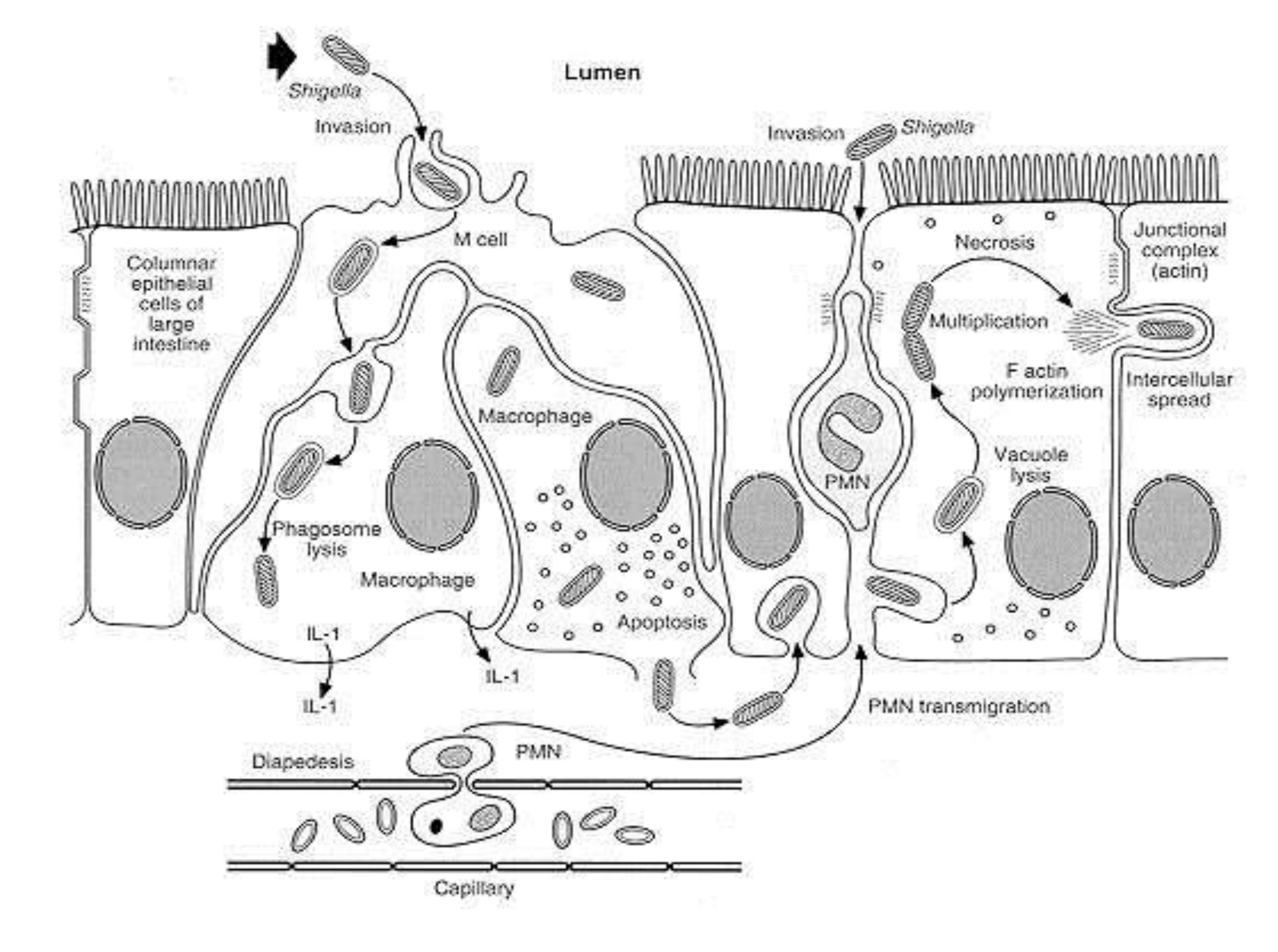
- It causes local areas of erosion that give rise to bleeding and heavy mucous secretion. The toxin also leads to nerve cell damage. Shiga toxin is composed of A (enzymatic) and B (binding) subunits in a ratio of 1:5. One component binds to the host cell surface, while the other passes into the cell membrane or cytoplasm before acting.

Shiga Toxin Effects in Shigellosis
Enterotoxic Effect:

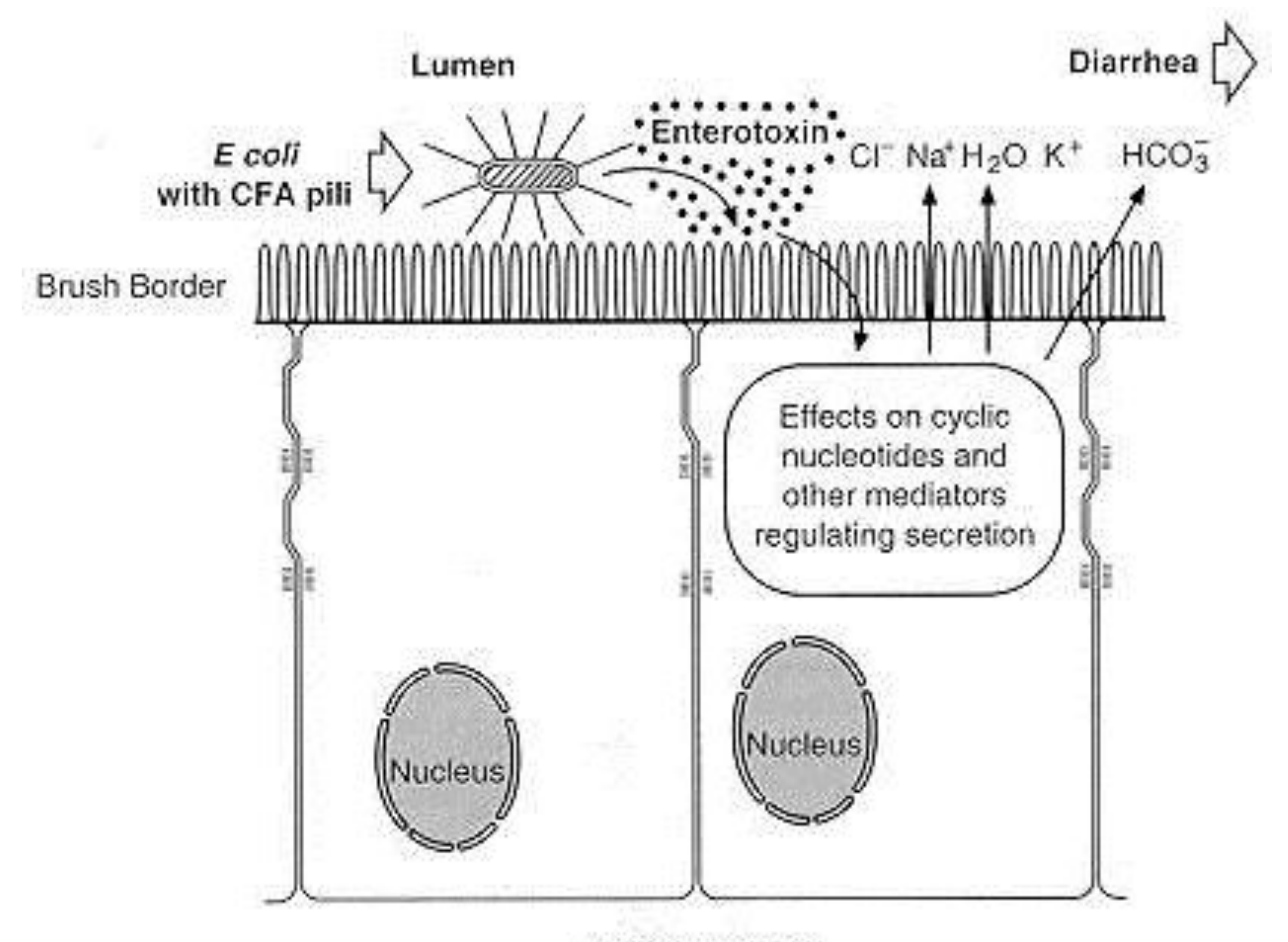
Adheres to small intestine receptors Blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen

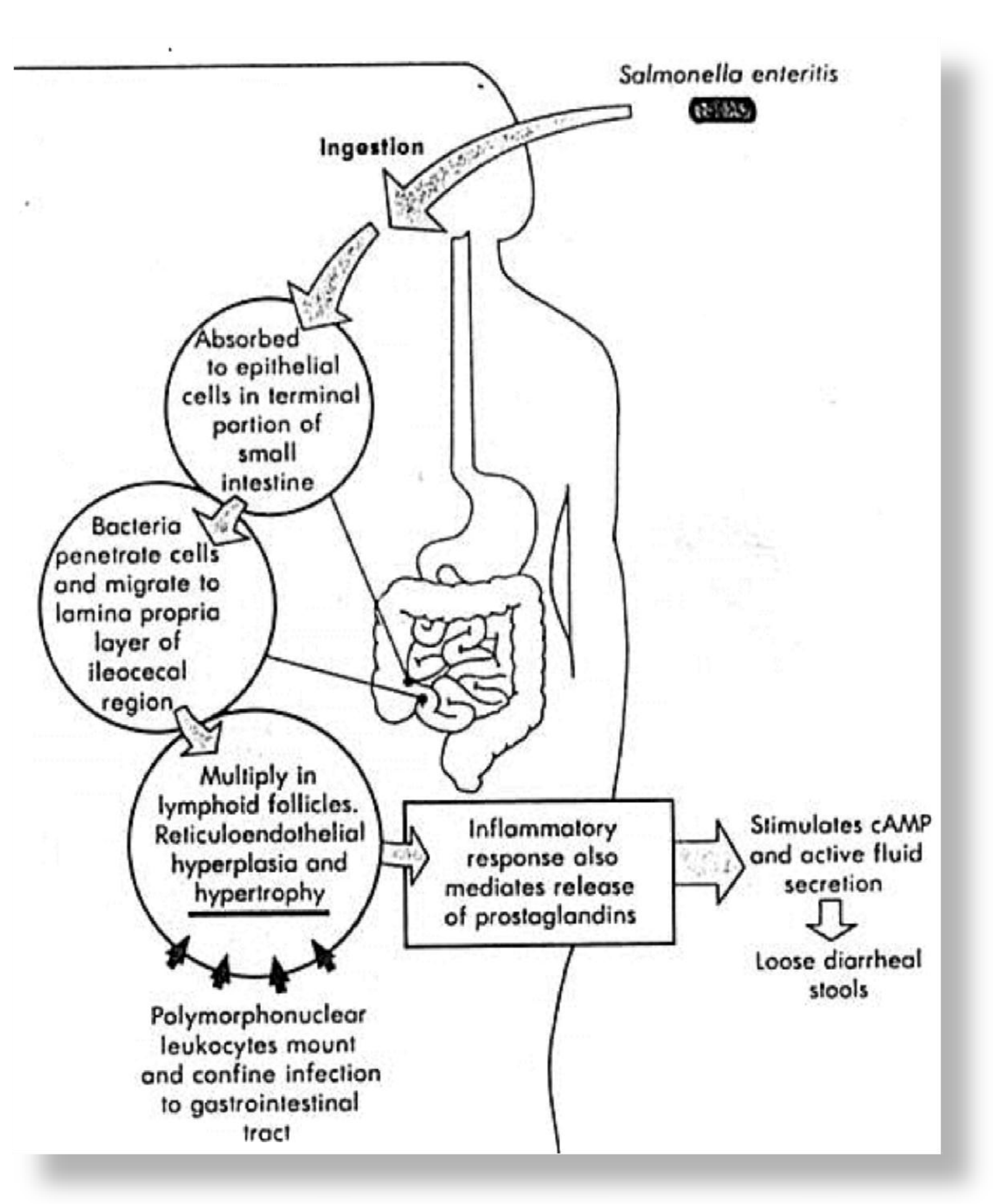
Note:

- This contrasts with the effects of cholera toxin (*Vibrio cholerae*) and labile toxin (LT) of enterotoxigenic *E. coli* (ETEC) which act by blocking absorption of Na+, but also cause hypersecretion of water and ions of Cl^-, K^+ (low potassium = hypokalemia), and $HCO3^-$ (loss of bicarbonate buffering capacity leads to metabolic acidosis) out of the intestine and into the lumen



Lumen
Shigella
Invasion
M cell
Columnar epithelial cells of large intestine
Phagosome lysis
Macrophage
Macrophage
IL-1
IL-1
IL-1
Apoptosis
Invasion
Shigella
Necrosis
Junctional complex (actin)
Multiplication
F actin polymerization
Intercellular spread
PMN
Vacuole lysis
PMN transmigration
PMN
Diapedesis
Capillary


Lumen
Diarrhea
E coli
with CFA pili
Enterotoxin
Cl⁻ Na⁺ H₂O K⁺ HCO₃⁻
Brush Border
Effects on cyclic nucleotides and other mediators regulating secretion
Nucleus
Nucleus
Lamina propria



Salmonella

Exotoxins:

- Effects in host have not been identified Several Salmonella serotypes produce enterotoxins similar to both the heat-labile (LT) and heat-stable enterotoxins (ST), but their effect has not been identified.
- Inflammatory response mediates release of prostaglandins, stimulating cAMP and active fluid secretion with loose diarrheal stools; epithelial destruction occurs during late stage of disease